

AMENDMENTS TO THE CLAIMS

1. (currently amended) A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:

a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:

- (i) eukaryotic genomic DNA;
- (ii) a plurality of pairs of forward and reverse DNA primer

oligonucleotides wherein one primer of each said pair is complementary to a 3' sequence of a targeted segment of a first DNA strand of the eukaryotic DNA and the other primer is complementary to the 3' sequence of the second strand of the targeted segment, the length of the segment of eukaryotic DNA being between about 50 and about 300 base pairs, wherein one of the primers of each pair has a detectable label attached to its 5' end, and wherein a plurality of the pairs of primers are each targeted to a segment of a selected different chromosome of interest which is indicative of a potential chromosomal disorder and one pair is targeted for a segment of a single control gene which is present on a chromosome other than one on which there is a targeted segment and does not target any chromosome segment that might be indicative of a potential aneuploidy; and

iii. PCR buffers and enzymes necessary to carry out PCR amplification;

b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;

c. purifying said products of step (b) and obtaining single-stranded DNA having the detectable labels,

d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of each of said targeted segments;

e. hybridizing said DNA oligonucleotide probes and said PCR-amplified label-containing single-stranded products;

f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by imaging the microarray; and

g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said selected different chromosomes by comparing said imaging of the relevant spots on said microarray for each said targeted segment of a selected chromosome to the imaging of spots relevant to said single control gene and then to results obtained from similar testing of genomic DNA known to be normal.

2. (original) The method according to claim 1 wherein said step of diagnosing includes the application of rule-based algorithms to the detection results of step (f) prior to final comparison to said results for normal genomic DNA of the same gender.

3. (original) The method according to claim 1 wherein at least two of the targeted segments of eukaryotic genomic DNA selected are associated with potential microdeletions of chromosomal DNA that would give rise to chromosomal disorders selected from the group consisting of:

Williams-Beuren syndrome,

Cri du chat syndrome, and

DiGeorge syndrome.

4. (original) The method according to claim 1 wherein at least two of the targeted segments are selected to detect chromosomal aberrations selected from the group consisting of trisomy 13, trisomy 18, trisomy 21 and X- and Y-chromosome anomaly.

5. (original) The method according to claim 1 wherein said detectable labels are color-detectable labels.

6. (original) The method according to claim 5 wherein said color-detectable labels are attached to the reverse primers and the forward primer of each pair has phosphate at its 5 end.

7. (original) The method of claim 6 wherein said detectable labels are fluorescent dyes.
8. (original) The method of claim 1 wherein the double-stranded product of step (b) is first purified and then the sense strands of the purified product are digested with an exonuclease to obtain the single-stranded labeled antisense strand in step (c).
9. (currently amended) The method of claim 1 wherein the said single control gene is GAPD.
10. (original) The method of claim 1 wherein the sizes of the probes range from about 25 to about 60 nucleotides and the targeted segments are between about 100 and 200 base pairs long.
11. (original) The method of claim 1 wherein two microarrays are used in parallel and the imaging results from both are compared as an initial check on the validity of the hybridizing and imaging steps.
- 12-19. (canceled)
20. (currently amended) A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:
 - a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:
 - (i) eukaryotic genomic DNA;
 - (ii) a plurality of pairs of forward and reverse DNA primer oligonucleotides wherein one primer of each said pair is complementary to a 3' sequence of a targeted segment of a first eukaryotic DNA strand and the other primer is complementary to the 3' sequence of the second strand of the target segment, the length of the segment of eukaryotic DNA being between about 100 and about 250 base pairs, wherein one of the primers of each pair has a color-

detectable label attached at the 5' end thereof, and wherein a plurality of the pairs of primers are targeted to segments of selected different chromosomes of interest which are indicative of potential chromosomal disorders and one pair is targeted for a segment of a single control gene; and

- (iii) PCR buffers and enzymes necessary to carry out PCR amplification;
- b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;
- c. purifying said products of step (b) and obtaining single-stranded DNA having the color-detectable labels by digestion of one strand of the amplified double-stranded PCR product,
- d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of said targeted segments;
- e. hybridizing said DNA oligonucleotide probes and said PCR-amplified label-containing single-stranded products;
- f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by colorimetric imaging of the microarray; and
- g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said different chromosomes of interest by first comparing said imaging of a relevant spot on said microarray for each said chromosome of interest to the imaging of a spot relevant to said single control gene to obtain an I- ratio; then comparing each I-ratio to N-ratios that have been obtained as a result of similar testing of genomic DNA known to be normal, said N-ratios being averages for normal DNA of persons of that gender, of the ratios of intensity of each said segment for each said chromosome of interest to the intensity of said single control gene.

21. (currently amended) The method according to claim 20 wherein said I-ratios are subjected to rule-based algorithms so that involves the adjustment of each I-ratio is adjusted prior to its use in final diagnosis by using an average C-factor which is obtained after first comparing all of the I-ratios with the respective N-ratios to obtain individual C-factors.